REMARKS

Status of the Claims

Claims 15, 18 and 19 are pending in the application. Claims 15, 18 and 19 are rejected. Claims 15 and 18 areamended herein. No new matter is added.

Claim amendments

Claims 15 and 18 areamended to overcome the 35 U.S.C. §112, first paragraph rejection. Amended claim 15 is drawn to a method of diagnosing DKK1-associated lytic bone disease in an individual. This method comprises measuring the expression of the human homologoue of Dickkopf-1 (DKK-1) protein in the individual, where an increased expression of the protein compared to that in a healthy individual indicates that the individual has the risk of developing lytic bone disease (page 5, lines 7-21; Table 1; Example 9; Example 10; page 6, lines 1-4 and lines 17-21, Figure 8, and Figure18). Amended claim 18 recites methods of measuring DKK1 expression level.

Claim rejection under 35 U.S.C. §112

Claims 15, 18 and 19 are rejected under 35 U.S.C. §112, second paragraph as being indefinite. The Examiner states claims 15 and 19 are incomplete for omitting essential steps. In particular, the Examiner states, that it is

unclear as to what steps are involved in the examination of the level of human homologue of Dickkopf-1 protein.

Applicants submit amended Claim 15. Amended claim 15 is drawn to a method of diagnosing DKK1-associated lytic bone disease in an individual. Such a method comprises measuring the expression of the human homologoue of Dickkopf-1 (DKK-1) protein in the individual, where an increased expression of the protein compared to that in healthy individual indicates that the individual has the risk of developing lytic bone disease (page 5, lines 7-21; Table 1; Example 9; Example 10; page 6, lines 1-4 and lines 17-21). Further, amended claim 18 recites methods of measuring expression levels for DKK1. Applicants submit that, one skilled in the art can accomplish measuring the protein levels of DKK1 by known and standard methods in the art. The Examiner has not provided any scientific evidence to the contrary. These methods include but are not limited to ELISA, immunohistochemistry and flow cytometery. Hence, amended claims 15 and 18 clearly specify the steps involved in the diagnosing DKK1-associated lytic bone disease in an individual.

In view of the amendments and the arguments presented supra, Applicants respectfully request, that the rejection of claim 15 under 35 U.S.C. §112, second paragraph be withdrawn and since, claims 18 and 19 are dependent from claim 15 hence, Applicants submit that claims 18 and 19 are in condition for allowance.

Claims 15 and 18-19 stand rejected under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. In the instant case, the Examiner states the claims are inclusive of a genus of compounds referred to as "WNT Antagonists", which encompass a genus of molecules defined solely by its principal biological properties. Accordingly, the Examiner states that, there is insufficient written description encompassing lytic bone disease.

Applicants submit amended claim 15, which has been amended to remove the phrase "WNT antagonist associated" and instead recites DKK1-associated lytic bone disease. Further, the claim recites that the method of diagnosing said lytic disease, in an individual suffering from myeloma, is based on measuring and comparing, levels of the protein between an individual suffering from multiple myeloma to a normal individual. The instant specification supports the association between DKK1 expression and development of lytic bone disease. Since multiple myeloma is associated with lytic bone disease and local bone destruction, the instant invention examined the expression of 12,000 genes in plasma cells of newly diagnosed multiple myeloma patients with or without lytic bone lesions.

The instant specification teaches that two secreted Wnt signaling antagonists, SFRP-3/FRZB and DKK-1 were expressed in 40 of 47 plasma cells of multiple myeloma patients with lytic bone lesions and in only 16 of 28 plasma cells of multiple myeloma patients lacking bone lesions. Importantly, DKK-1 and FRZB were not expressed in plasma cells from 45 normal bone marrow donors of

Waldenstrom's macroglobulinemia, a plasma cell malignancy that lacks bone disease (page 5, lines 7-21; Table 1; Examples 1, 9, 10).

The levels of expression of these genes were also consistent with the expression profile when examined immunohistochemically (Example 15). Additionally, the instant invention also disclosed that serum derived from multiple myeloma patients with high DKK-1 blocked both Wnt signaling and osteoblast differentiation in vitro and that pre-incubation of serum with DKK-1 and FRZB antibodies inhibited this function (page 6, lines 1-4). The instant invention further teaches that the DKK-1 and FRZB inhibitors can be used to prevent bone loss in general population (page 6, lines17-21).

Further, the specification of the instant invention clearly states and the data presented therein demonstrates that the secreted Wnt-signaling antagonists, DKK-1 mediates bone destruction seen in multiple myeloma. This along with the emerging evidence of an absolute requirement of Wnt-signaling in osteoblast growth and differentiation strongly implicate these factors in causing osteoblast anergy and contributing to multiple myeloma bone disease by suppressing the normal compensatory bone production that follows bone loss (page 17, line 16-page 18, line 2). The secreted DKK-1 and FRZB could account for both the systemic osteoporosis seen in multiple myeloma as well as the exaggerated local bone destruction proximal to plasma cells foci (page 18, lines 9-12). Thus, these genes could be used to predict extent of bone disease and future risk of developing bone disease (page 18, lines 15-18). Furthermore, the instant specification also discusses a model that shows how DKK-1 expression by multiple myeloma plasma cells can be linked to multiple

myeloma disease growth control and bone destruction (page 23, line 9-page 24, line 17).

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosure in the patent coupled with the information known in the art without undue experimentation (M.P.E.P. 2164.01). As discussed supra, Applicant submits that the instant specification provides sufficient enablement for using the claimed method to diagnose individuals at risk of developing DKK1-associated lytic bone disease. Thus, the scope of the claimed invention is commensurate with the enablement provided. Based on the above-mentioned amendments and remarks, Applicant respectfully requests the withdrawal of rejection of claims 15, 18 and 19 under 35 U.S.C. §112, first paragraph.

Claims 15 and 18-19 stand rejected under 35 U.S.C. §112, first paragraph. The Examiner states, that while the specification is enabling for a method of diagnosing a DKK1-associated lytic bone disease in an individual having multiple myeloma, it does not provide enablement for a method of diagnosing any and/or all Wnt antagonist- associated lytic bone disease in any test individual comprising examining the expression level of the human homologue of Dickkopf-1 (DKK1) protein in said individual.

Applicants submit amended Claim 15, which is drawn to a method of diagnosing DKK1/associated lytic bone disease in an individual. The instant specification teaches the importance of Wnt signaling pathway in bone development (page 19, 20, 21, and 22). The Wnt family of secreted growth factors initiate signaling

via the Frizzled (Fz) receptor and its co-receptors, LDL receptor-reLated protein 5 or 6 (LRP5 or LRP6). Targeted disruption of LRP5 (i.e., disruption of Wnt-signaling) in mice, results in low bone mass phentype. This phenotype becomes evident post-nataly and is secondary to osteoblast proliferation. Further, in humans loss of function mutations in LRP5 causes the autosomal recessive disorder osteoporosis-pseudoglioma syndrome. Individuals with osteoporosis-pseudoglioma syndrome have reduced bone mass as compared to age and gender matched controls. In contrast to loss of function mutations, gain of function mutations in LRP5 reveal markers of bone formation like osteocalcin to be highly elevated. FRZB and DKK1 are two of the secreted antagonists of Wnt. DKK1 specifically inhibits Wnt signaling by binding to LRP5/LRP6 component of the receptor complex. Normal inhibition of Wnt signaling by DKK1 is defective in presence of gain of function mutations in LRP5. Thus, the increase in WNT signaling mediated by decrease in DKK-1 inhibition results in higher osteoblast activity leading to high bone mass.

This unopposed Wnt signaling, resulting in high bone mass, provides evidence for inhibition of DKK1 as a target for the prevention or treatment of osteoporosis. In addition, targeted disruption of secreted frizzled-related protein (SFRP-1), a homologue of FRZB, leads to decreased ostoblast and osteocyte apoptosis and increased trabecular bone formation. These studies further present evidence for the role of FRZB in development of lytic bone disease. Furthermore, the instant specification teaches that the expression of the two secreted Wnt signaling antagonists, SFRP-3/FRZB and DKK-1 in multiple myeloma patients was linked with development of lytic bone lesions (examples 8 and 9). One can logically conclude

from above, that if the level of DKK-1 expression increases then there would be low osteoblast (bone formation) activity due to a concomitant decrease in WNT signaling. Thus an individual exhibiting higher levels of DKK-1 protein would be at a high risk of developing a bone disease characterized by bone loss as a result of low osteoblast activity that is mediated via the WNT signaling pathway.

Applicants submit that Example 17 further provides important data on the role of DKK-1 in osteoblast differentiation. Bone morphogenic protein-2 can induce differentiation of uncommitted mesenchymal progenitor cell line, C2C12, into osteoblats through a mechanism that involves WNT/β-catenin signaling. Alkaline phosphatase, a specific marker of osteoblast differentiation was inhibited in C2C12 cells cultured with BMP-2 and bone marrow serum with a DKK-1 concentration >12 ng/ml (from donors with multiple myeloma). This inhibition was also observed in the presence of 50 ng/ml of recombinant human DKK-1. A reversal of alkaline phosphatase inhibition was observed in the presence of anti-DKK-1 antibody. The inhibition of alkaline phosphate was not seen when the bone marrow serum from a normal donor was used (Figure 41B). This clearly demonstrates the inhibitory effect of DKK-1 on osteoblast activity.

Further, the Applicants respectfully submit a Declaration by the inventor, Dr. John Shaughnessy that unquestionably supports the critical role of DKK1 in bone remodeling in adults. Dr. Shaughnessy Declaration clearly demonstrates that the application of a neutralizing antibody directed to DKK1 results in an increase in bone mineral density of non-myelomatous bones, *in vivo* (nonmyelomatous SCID-RAB model), thereby clearly demonstrating bone anabolic

effects, as a result of blocking DKK1, are not limited only to myeloma affected bones but also occur in non-myeloma affected bones.

In view of the plethora of evidence linking DKK1 to bone development, there exists no unpredictability associated with correlating the expression level of DKK-1 with the ability to provide diagnostic evaluation of a patient suspected of lytic bone disease. Given the teachings of the instant specification, one skilled in the art can easily diagnose an individual suffering from DKK1-associated lytic bone diseases by comparing the expression level of DKK-1 protein of such individuals with the expression of the protein of healthy individuals. An aberrantly high expression of DKK1, as compared to the levels observed in the normal/healthy individual would diagnose said individual with lytic bone disease.

Applicants present amended claim 18, which recites the methods of measuring DKK1 expression. The techniques and methods for measuring expression levels of proteins are existent and standard in the art. Hence, no undue experimentation would be required for measuring the protein levels. Further, the instant invention teaches measuring expression of the protein as a method of diagnosis of lytic bone disease in an individual, for which there is adequate support in the instant specification, and not for the development of the protein as biomarkers for predicting bone disease. The instant invention links the aberrant expression of DKK1 to presence of lytic bone disease and not to the risk of developing bone disease. Establishing the presence of a specific factor, such as over expression of DKK-1, in individuals provides suitable clinical guidelines for diagnosis of such bone related diseases. Therefore, experimentation necessary in

bringing a disease biomarker to successful clinical applications do not apply to the instant invention.

In view of the arguments presented supra and the enclosed Declaration, Applicant submits that the instant specification provides sufficient guidance and exemplification correlating the level of DKK1 with the ability to provide diagnostic evaluation of a patient suspected of exhibiting lytic bone disease. Applicant respectfully submits that "the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without undue amount of experimentation. Because only an enabling disclosure is required, Applicant need not describe all actual embodiments" (M.P.E.P. 2164.02). In this case the instant invention encompasses, diseases manifesting bone loss, associated with overexpression of DKK1 (DKK1-associated bone loss). Thus, the scope of the claimed invention is commensurate with the enablement provided. Based on the above-mentioned amendments and remarks, Applicant respectfully requests that the rejection of claims 15 and 19 under 35 U.S.C. §112, first paragraph be withdrawn.

Double Patenting Rejection

Claims 15 and 18-19 are provisionally rejected on the grounds of nonstatutory obviousness double patenting as being unpatentable over claims 1-3 of copending Application No. 10/176,739.

If necessary, Applicants will submit a terminal disclaimer in compliance with 37 CFR 1.321(C) or 1.321 (d) to overcome this provisional nonstatutory double patenting rejection.

This is intended to be a complete response to the Office Action mailed September 14, 2006. Applicant submits that the pending claims are in condition for allowance. If any issues remain outstanding, please telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

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